bio-protocol Preprint

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par-seqFISH

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An abbreviated version of this protocol was published in Science in Aug 2021

Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution

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Detailed protocol

Making solutions:

Primary hybridization buffer (1.67x for a 50% final)

UltraPure SSC 20X		Gibco LifeTech 15557-036	1.67 ml
Formamide		Ambion AM9342 (ThermoFisher)	8.35 ml
Dextran sulfate **HW**		Sigma-Aldrich D8906-5G	1.67 g
			10 ml

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Primary and secondary wash buffers

10% wash buffer / *55% wash buffer*

UltraPure SSC 20X	Gibco LifeTech 15557-036	1 ml
Formamide	Ambion AM9342 (ThermoFisher)	1 ml / ***5.5ml***
TritonX-100 (10%)	Sigma-Aldrich 93443	100 ul
UltraPure water	IDT 11-05-01-04	7.9 ml / ***3.4ml***
		10 ml

Secondary hyb buffer: 10% EC (1.67x for a 10% final)

50%(v/v) EC solution

100% EC solution	6 mL
Ultrapure water	6 mL

10% (v/v) EC solution

UltraPure SSC 20X	Gibco LifeTech 15557-036	6 ml
50% EC solution	What you just made above	12 mL
Dextran sulfate **LW**	Sigma-Aldrich D4911-10G	6 g
UltraPure water	IDT 11-05-01-04	~18 ml
		36 ml

Imaging solution:

Anti-bleaching buffer base (high salt)

Tris-HCl pH = 8.0 1M	Fisher Scientific J22638AE	3.575 ml
NaCl 5M	Thermo Fisher AM9759	4.29 ml
Trolox (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid)	Sigma 238813-1G	~0.0375 g
UltraPure water	IDT 11-05-01-04	~42.1 ml
		50 ml

Assembling the solution:

Ab buffer (above)	Above	70 ul
D-Glucose (make 8% from 20% - in UltraPure water- and use the 8%)	Sigma-Aldrich 49163	10 ul
Catalase (use 100x = 10-fold dilution of original)	Sigma-Aldrich C3155-50MG	1 ul
Glucose oxidase (prepare a 0.05g in 1ml H20 for a 100X solution)	Sigma-Aldrich G2133	1 ul
20x SSC	Gibco LifeTech 15557-036	10 ul
UltraPure water	IDT 11-05-01-04	8 ul
		100 ul

Preparation of PDL + Bind silane coated slides:

It is recommended to treat the coverslips with plasma cleaner. However, its also possible to get good results without it.

- Place coverslips in slide holder and fill with 100% EtOH to cover the coverslips completely
- Remove access EtOH and place in oven to dry completely (about 5-10min)
- Place in 1% Bind silane for 1h RT
    - 180 ml EtOH (100%), 2ml 1M Acetic Acid, 16ml RNAse free water, 2 ml Bind Silane.
- Wash 3 times in EtOH
- Heat in oven for 30 mins 90C
- Place ~400uL of PDL solution (PDL 100µg/ml in 1X PBS) on each coverslip and let it sit O.N RT
- Remove solution gently.
- Wash with RNAse free water twice
- Remove water and let coverslips dry (can also dry with compressed air)
- Attach coverslips to the glass-slides by placing a drop of ethanol on the slide and putting the coverslip on it (coated side up-this is where the tissue will be)
- Store in (-20)

Acrylamide based gel coating

Preparing APS and TEMED solutions

- APS -> 0.2g in 800ul H20
- 4ul TEMED + 16ul H20
- Both on ice until use

Preparing Initiator solution

- Make 25% VA044 (Wako Chemicals) by mixing 0.125g + 400ul H20
- Can keep for a few days in 4c

Prepare Acrylamide (AA) solution: dilute 30% -> 4%

- 133.3 ul of 30% Acrylamide/Bis Solution, 19:1
- 100ul 20x ssc
- 766.6 H20 (complete to 1ml)

Degas 4% AA (remove O2)

Degas 4% AA (remove O2)

- Make 2 holes in Eppendorf tubes using a need
- Insert needle with N2 and leave one hole for output
- Needle directly into solution.
- Run N2 10min
- Close holes with sticker
- Place on ice

Prepare the gel mixture (Mix on ice)

- 958 ul 4% AA
- 40 us VA 25% (initiator)
- 1ul APS
- 1ul TEMED

Casting the gel

- Place a 0.1 mm donut shaped sticker / chamber around your sample. You can stack a few if needed.
- Cover sample with gel mixture and place a slide to weight it down.
- Place in a tip box chamber and degas again for 15min (make 2 holes in top and insert N2)
- Close the holes
- Incubate at 37c for 1-3 hours.
- Gently remove the slide without ripping away the gel and your sample.

The reactions

Mounting cells on coverslips

- Place a drop of fixed and permeabilized bacteria (5-10ul) on the coverslip
- Let sit on bench for 5 min
- Centrifuge coverslip 500g-1000g for 5min
- Place a hybridization chamber

Primary probe hybridization

1. Assemble reaction mixture and mix well -> insert in to hyb chamber
2. Incubate at 37°C for ~16h (best done in a humidified chamber)
3. Gently remove hyb buffer by pipetting
4. Wash twice with 55% formamide wash buffer.
5. Fill chamber with 55% wash buffer and incubate for 30min at 37°c (or RT).
6. Wash 3 times with 2x SSC buffer.

Example reaction		(ul)
Buffer (50% formamide 1.67x stock)		30
Primary probes (4nM final)		2
H_20		18
total		50

Secondary probe hybridization

1. Assemble reaction and add mixture to the sample.
2. Incubate at RT for 20 min in dark
3. Wash twice with a 10% wash buffer.
4. Fill chamber with 10% wash buffer and incubate for 5 min.
5. Wash twice with 2x SSC.
6. Optional: add DAPI for 1 min to stain cells.
7. Replace with an imaging buffer (anti-bleaching) and keep in the dark until imaging.
8. Go take some images!

Example reaction		(ul)
Buffer (10% EC 1.67x stock)		30
Secondary probe (500nM stock)		5
H_20		15
total		50